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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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EXAMINER

RAWLINGS, S

ART UNIT

PAPER NUMBER

1642

DATE MAILED:

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.

09/559,874

Applicant(s)

LENG, JAY

Examiner

Stephen L. Rawlings, Ph.D.

Art Unit

1642

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 08 February 2001.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-70 is/are pending in the application.
- 4a) Of the above claim(s) 48-62, 69, and 70 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-48 and 63-68 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☒ Claims 1-70 are subject to restriction and/or election requirement.

Application Papers

- 9) ☒ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- 11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved.
- 12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- 13) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
 - ☐ Certified copies of the priority documents have been received in Application No. _____.
 - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.
- 14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- 15) ☒ Notice of References Cited (PTO-892)
- 16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 17) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____
- 18) ☐ Interview Summary (PTO-413) Paper No(s). _____
- 19) ☐ Notice of Informal Patent Application (PTO-152)
- 20) ☐ Other:

DETAILED ACTION

1. Applicant's election of Group I, claims 1-47 and 63-68 in Paper No. 6 filed on February 9, 2001 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

2. Claims 1-70 are pending in the application. Claims 48-62, 69, and 70 have been withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention. Claims 1-47 and 63-68 are currently under prosecution.

Specification

3. The disclosure is objected to because of the following informalities:

On page 14, the ATCC accession number in line 12 has been omitted. Appropriate correction is required.

On page 19, "coelentraine" occurs twice in line 2 and appears to be a misspelling of the word "coelenterazine".

Claim Rejections - 35 USC § 112

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 1-30 and 63-68 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 1-17 are drawn to a method for determining the effect of an agent on cell proliferation, said method comprising comparing the light emission data from the cell

containing a *Renilla* luciferase polypeptide or a polynucleotide encoding a *Renilla* luciferase. Claims 18-30 are drawn to a method for determining cell proliferation of a cell or population of cells, said method comprising obtaining light emission data from the cell. Claims 31-47 are drawn to a method for determining the effect of an agent on cell proliferation, said method comprising transfecting a cell obtained from a sample with a vector encoding *Renilla* luciferase and comparing the light emission data from the cell. Claims 63-68 are drawn to a method of screening mammalian cells to determine their susceptibility to treatment with an agent, said method comprising measuring light emissions from the cells.

The specification teaches that coelenterazine is the substrate utilized in the enzymatic reaction by the *Renilla* luciferase to generate light (page 19, line 28 to page 20, line 4). The specification teaches in the examples on pages 24-26 that a cDNA molecule encoding *Renilla* luciferase can be cloned, that the vector carrying the cloned cDNA molecule encoding *Renilla* luciferase can be transfected into mammalian cells, including HeLa cells, and that the presence of *Renilla* luciferase in lysates derived from the transfected cells can be determined. In Figures 2 and 3, the specification teaches that the proliferation of the transfected cells can be monitored by repeatedly measuring the light emitted by the lysates over a period of time. The specification discloses that luciferase activity (i.e., light emission) can be measured either *in vivo* (i.e., from within intact cells or from within the body) or *in vitro* (i.e., from within the extracts of lysed, isolated cells) (page 19, lines 29-30). Accordingly, the specification also discloses that the proliferation of cells in a human subject can be monitored using the claimed invention (page 21, lines 10-13).

The teachings of the specification cannot be extrapolated to the enablement of the invention commensurate in scope with the claims, because the guidance and exemplification in the specification is insufficient to enable one skilled in the art to use the invention with a reasonable expectation of success. The reasons this conclusion are made are set forth below:

Art Unit: 1642

Dubuisson, et al teach that the substrate, which is called coelenterazine, utilized by *Renilla* luciferase in the enzymatic reaction that generates the light that is emitted and measured in the claimed method is a strong toxin (abstract). Specifically, Dubuisson, et al disclose that coelenterazine was highly toxic to mammalian liver cells (page 473, column 2). Notably, the level of toxicity was even more accentuated in the presence of a drug called *tert*-butyl hydroperoxide (abstract).

On the basis of the teachings of Dubuisson, et al one of skill in the art would immediately recognize that the data collected in the process of practicing the claimed invention may be invalidated by the detrimental effects of the substrate on cell proliferation. Because of the fact that coelenterazine is toxic to mammalian cells, one of skill in the art cannot predict whether the invention can be used to successfully monitor cell proliferation and the specification is silent as to how valid light emission data can be collected in practicing the claimed methods. In regard to the claims drawn to methods for determining the effect of an agent on cell proliferation and for screening mammalian cells to determine the susceptibility to treatment with an agent, one of skill in the art cannot predict whether the magnitude of the effect is entirely dependent upon the toxicity of the antibiotic or chemotherapeutic agent that is being tested or screened. Because the contribution made by the inherent toxicity of coelenterazine to the cumulative effect on cell proliferation cannot be ascertained from the teachings in the specification, one skilled in the art would not be able to validate the collected light emission data or to practice the invention with a reasonable expectation of success.

With the exceptions of claims that recite that the light emissions from cells *obtained from a sample or a subject* are to be measured or compared (i.e., claims 27-47, 64, and 65), the scope of the invention is reasonably interpreted to encompass methods that can be practiced using cells in a human subject. Of course, since it seems unlikely that one would find HeLa cells in a human subject, claims 8 and 26, which recite the limitation that the cell is a HeLa cell, are not drawn to the breadth of scope that would include cells in a human subject. Because of the inherent toxicity of coelenterazine, clearly one skilled in the art would not administer the substrate to a subject, particularly not a human subject in order to practice the invention, without

Art Unit: 1642

approval by the Food and Drug Administration (FDA). Coelenterazine has not been approved for use in humans. Dubuisson, et al teach another substrate, methyl coelenterazine, which presumably can be used in place of coelenterazine in the claimed method that is not toxic (page 473, column 2). However, the specification does not teach the use of this or any other alternative substrate that is nontoxic or approved by the FDA for use in humans.

Additionally, because the drug, *tert*-butyl hydroperoxide, increases the toxicity of coelenterazine, it is also reasonable to presume that other agents will also result in a greater level of cytotoxicity (see Dubuisson, et al, cited supra). Clearly, because some agents may have an additive or synergistic effect and amplify the inherent toxicity of coelenterazine, the analysis of data collected by the claimed method of screening mammalian cells for susceptibility to such agents and the claimed method for determining the effect of an agent on cell proliferation is complicated. Claim 1 requires that the agent be "suspected of modulating cell proliferation under conditions that allow the agent and the cells to interact". However, if the agent amplifies the toxicity of coelenterazine but, in and of itself, the agent is nontoxic, then the use of the claimed method will result in an incorrect determination that the agent is effectual. According to claim 17, the agent can be suspected of stimulating cell proliferation. However, if the positive effect of the agent is counterbalanced by the negative effect of coelenterazine on cell proliferation, clearly the claimed method will be ineffectual. Also, claim 18 recites that "a change in light emission data is indicative of proliferation". However, this correlative statement appears to be an inaccurate, because a decrease in light emission is not indicative of the cell proliferation; rather, it may be indicative of the fact that cells are dying. Furthermore, it is apparent from the claims that the invention encompasses a method of screening potential chemotherapeutic agents that might be useful for treating cancer in patients, where such agents cause a decrease in cell proliferation or the rate of tumor growth. In regard to this issue, Cree (*Methods in Molecular Biology* **102**: 169-177, 1998) teach the following on page 170 (paragraph 1):

Any method that allowed the chemosensitivity of tumors to be predicted in individual patients would be welcome, since it would allow optimal treatment to be given to each

Art Unit: 1642

patient. Many attempts have been made to do this, but no chemosensitivity test has yet achieved widespread clinical use (7,8). Prediction of chemosensitivity on the basis of tumor growth rates, estimated by histological methods or by nucleotide incorporation has proven disappointing. It is unlikely that molecular methods will fare much better, since the response to chemotherapeutic agents is determined by a large number of different biochemical pathways.

Thus, in view of the teachings of Cree, there is an unacceptable degree of unpredictability associated with interpreting the results of a molecular method, such as that claimed in the instant application. Moreover, Jassim, et al (*Journal of Bioluminescence and Chemiluminescence* 5: 115-122, 1990) teach that "in all instances, a key requirement for the application of bioluminescence is the establishment of a strict correlation between *in vivo* bioluminescence and cell viability" (abstract). However, the specification has not established that such a strict correlation exists. Yet another problem with the invention is evident from the teachings of US 6,171,809 B1, which discloses that "the use of renilla luciferase as a reporter has been limited by the short period of light generation" (column 1, lines 36-38). The specification teaches no remedy to the problem nor does it emphasize that measurements must be acquired rapidly or that there is a potential limit to the time in which the measurements made will be valid. Because the specification provides insufficient guidance in regard to these issues, one skilled in the art would be forced into undue experimentation in order to practice the invention with a reasonable expectation of success.

Furthermore, it is noted that the specification does not exemplify the claimed method for screening mammalian cells to determine their susceptibility to treatment with an agent and it is unclear from the specification how the susceptibility to treatment with an agent is related to cell proliferation. Moreover, there are no working examples that teach the use of the claimed method for determining the effect of an agent on cell proliferation. Also, while the specification teaches a method for determining cell proliferation over time in a culture of mammalian cells (e.g., transfected HeLa cells), it does not exemplify a method for determining prokaryotic cell proliferation. The specification does not teach how, or which methods can be used to obtain samples from a subject. Where those samples are tissue sample, the specification does not teach

Art Unit: 1642

methods for acquiring individual cells. Where those samples are biological samples, the specification does not teach methods for isolation and preparation of individual cells from blood, urine, or stool. Finally, the specification does not exemplify any method in which light emission from intact cells is measured; the only example teaches that a cellular lysate must be prepared and that luciferase activity is measured *in vitro*.

Because the specification provides insufficient guidance with regard to the issues set forth above and insufficient exemplification, one of skill in the art cannot practice the claimed invention with a reasonable expectation success without undue experimentation.

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claims 1-47 and 63-68 rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01.

The omitted steps in claims 1-17 are: (a) a step in which a cellular lysate is prepared, (b) a step in which coelenterazine is added to the lysate, and (c) a step in which the light emission data is collected from a sample of cellular lysate from cells in the presence and absence of an agent.

The omitted steps in claims 18-30 are (a) a step in which a cellular lysate is prepared, (b) a step in which coelenterazine is added to the lysate, and (c) a step in which the light emission data is collected at different time points is compared.

The omitted steps in claims 31-47 are: (a) a step in which a cellular lysate is prepared, (b) a step in which coelenterazine is added to the lysate, and (c) a step in which the light emission data is collected from a cellular lysate from cells in the presence and absence of an agent.

The omitted steps in claims 63-68 are: (a) a step in which a cellular lysate is prepared, (b) a step in which coelenterazine is added to the lysate, and (c) a step in which the light emission data is compared.

Note: Because the specification does not exemplify a method of measuring light emission directly from cell and only teaches an example in which light emission from cellular lysates is measured (see the 35 USC § 112, first paragraph enablement rejection above), the step in which a cellular lysate is prepared is considered an essential method step.

8. Claims 1-47 and 63-68 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-17 are indefinite because claim 1 recites the phrase "an agent" in line 3. The claim is drawn to a method of determining the effect of an agent on cell proliferation. However, because of the use of the indefinite article in line 3, it is unclear whether the effect on cell proliferation caused by contacting a cell with the agent of line 1 or the effect on cell proliferation caused by contacting a cell with some other agent is to be determined in the claimed method. Amending claim 1 to delete the indefinite article in line 3 before the word "agent" and to insert a definite article can obviate this rejection.

Claims 18-30 are indefinite because claim 18 recites the phrase "a cell" in line 3. The claim is drawn to a method of determining cell proliferation of a cell or a population of cells. However, because of the use of the indefinite article in line 3, it is unclear whether cell proliferation of the cell or population of cells in line 1 or cell proliferation of some other cell or population of cells is to be determined in the claimed method. Amending claim 18 to delete the indefinite article in line 3 before the word "cell" and to insert a definite article can obviate this rejection.

Claims 31-47 are indefinite because claim 31 recites the phrase "an agent" in line 5. The claim is drawn to a method of determining the effect of an agent on cell proliferation. However, because of the use of the indefinite article in line 5, it is unclear whether the effect on cell proliferation caused by contacting the transfected cell with the agent of line 1 or the effect on cell proliferation caused by contacting the transfected cell with some other agent is to be determined in the claimed method. Amending claim 31

Art Unit: 1642

to delete the indefinite article in line 5 before the word "agent" and to insert a definite article can obviate this rejection.

Claims 63-68 are indefinite because claim 63 recites the phrase "cells" in line 3. The claim is drawn to a method of screening mammalian cells to determine their susceptibility to treatment with an agent. However, because of the use of the indefinite article in line 3, it is unclear whether the susceptibility of the mammalian cells in line 1 or the susceptibility of some other cells is to be determined in the claimed method. Amending claim 63 to insert a definite article before the word "cells" in line 3 can obviate this rejection.

Claims 63-68 are also indefinite because claim 63 recites the phrase "an agent" in line 3. The claim is drawn to a method of screening mammalian cells to determine their susceptibility to treatment with an agent. However, because of the use of the indefinite article in line 3, it is unclear whether the susceptibility of mammalian cells to the agent of line 1 or the susceptibility of mammalian cells to some other agent is to be determined in the claimed method. Amending claim 63 to delete the indefinite article in line 3 before the word "agent" and to insert a definite article can obviate this rejection.

Claims 63-68 are also indefinite because it is unclear what relationship exists between cell proliferation and susceptibility to treatment in claim 63. It is not evident from the teachings of the specification that there is a correlation between cell proliferation and susceptibility to treatment. Also, the claim does not clearly indicate how the measurement of a difference in light emissions, which according to the claim is indicative of an agent that affects cell proliferation, results in the determination of a mammalian cell's susceptibility to treatment with an agent. Without affirming that the invention claimed therein is enabled, the amendment of claim 63 to delete the last two lines and insert the phrase "wherein a difference in light emissions is indicative of the cells' susceptibility to treatment with the agent" can obviate the claim. *rejection*

Claim Rejections - 35 USC § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

Art Unit: 1642

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

10. Claims 1-47 and 63-68 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cree (*Methods in Molecular Biology* **102**: 169-177, 1998) in view of Virta, et al (*Antimicrobial Agents and Chemotherapy* **38**: 2775-2779, 1994), Edinger, et al (*DRU* **1**: 303-310, 1999), Prosser, et al (*Critical Reviews in Biotechnology* **16**: 157-183, 1996), and further in view of US 5,292,658 A, and US 6,171,808 B1.

Claims 1-17 are drawn to a method for determining the effect of an agent on cell proliferation, said method comprising contacting a *Renilla* luciferase polypeptide or a polynucleotide encoding a *Renilla* luciferase with an agent suspected of modulating cell proliferation and comparing the light emission data from the cell to the light emission data from the cell in the absence of the agent (claim 1), wherein the cell is a prokaryotic cell (claim 2), or wherein the cell is a eukaryotic cell (claim 3) wherein the eukaryotic cell is a mammalian cell (claim 4) wherein the mammalian cell is a human cell (claim 5), or wherein the cell is a cancer cell (claim 6), or wherein the cell contains a transgene encoding *Renilla* luciferase (claim 7) wherein the cell is a HeLa cell (claim 8), or wherein the agent is either a peptide, a protein, a chemical, a nucleic acid, a small molecule, or a biological agent (claim 9) wherein the chemical is a drug (claim 10) wherein the drug is either an antibiotic (claim 11) or a chemotherapeutic agent (claim 11), or wherein the cell is obtained from a subject (claim 12) wherein the subject is a mammal (claim 13) wherein the mammal is a human (claim 14), or wherein the modulation is either inhibition (claim 15) or stimulation (claim 16) of cell proliferation. Claims 18-30 are drawn to a method for determining cell proliferation of a cell or population of cells, said method comprising obtaining light emission data from a cell containing a *Renilla* luciferase over a period of time (claim 18), wherein the cell is a prokaryotic cell (claim 19), or wherein the cell is a eukaryotic cell (claim 20) wherein the eukaryotic cell is a mammalian cell (claim 21) wherein the mammalian cell is a human cell (claim 22), or wherein the cell is a cancer cell (claim 23), or wherein the cell is in a culture of cells

Art Unit: 1642

(claim 24), or wherein the cell contains a transgene encoding *Renilla* luciferase (claim 25) wherein the cell is a HeLa cell (claim 26), or wherein the cell is obtained from a subject (claim 27) wherein the subject is a mammal (claim 28) wherein the mammal is a human (claim 29), or wherein the cell is obtained from a tissue sample (claim 30). Claims 31-47 are drawn to a method for determining the effect of an agent on cell proliferation, said method comprising transfecting a cell obtained from a sample with a vector containing a polynucleotide sequence encoding *Renilla* luciferase, contacting the transfected cell with an agent, comparing the light emission data from the cell to the light emission data from the cell in the absence of the agent (claim 31), wherein the cell is a prokaryotic cell (claim 32), or wherein the cell is a eukaryotic cell (claim 33) wherein the eukaryotic cell is a mammalian cell (claim 34) wherein the mammalian cell is a human cell (claim 35), or wherein the cell is a cancer cell (claim 36), or wherein the cell is obtained from a subject (claim 37) wherein the subject is a mammal (claim 38) wherein the mammal is a human (claim 39), or wherein the cell is obtained from a biological sample (claim 40) wherein the biological sample is either a blood sample, a urine sample, a stool sample, or a tissue sample (claim 41), or wherein the agent is either a peptide, a protein, a chemical, a nucleic acid, a small molecule, or a biological agent (claim 42) wherein the chemical is a drug (claim 43) wherein the drug is either an antibiotic (claim 44) or a chemotherapeutic agent (claim 45), or wherein the modulating is either inhibition (claim 46) or stimulation (claim 47) of cell proliferation. Claims 63-68 are drawn to a method of screening mammalian cells to determine their susceptibility to treatment with an agent, said method comprising contacting cells containing a *Renilla* luciferase with an agent and measuring light emissions from the cells in the presence and absence of the agent (claim 63), wherein the cells are obtained from a subject (claim 64) wherein the subject is a human (claim 65), or wherein the agent is either a peptide, a protein, a chemical, a nucleic acid, a small molecule, or a biological agent (claim 66), or wherein the chemical is a drug (claim 67) wherein the drug is either an antibiotic or a chemotherapeutic agent (claim 68).

It is noted that the claimed methods are so related that in order to practice one method another must also be practiced. For example, to practice the method of

screening mammalian cells to determine their susceptibility to an agent, one would necessarily have to measure cell proliferation over a period of time and to determine the effect of the agent on cell proliferation. As another example, to practice the claimed method for determining the effect of an agent on the proliferation of prokaryotic cells, one would necessarily have to measure cell proliferation over a period of time.

Cree teaches a luminescence-based cell viability assay, which can be used to measure cell proliferation and to test the effect of drugs on cell viability in order to screen candidate chemotherapeutic agents that might be used to treat cancer (page 169, paragraph 2). Cree teaches that cell lines can be used in this assay, because cell lines "provide a simple and convenient supply of cells suitable for ATP assays of toxicity or growth enhancement" (page 170, paragraph 3); in other words, the assay can be used to test the effects of agents that are suspected of modulating cell proliferation by either inhibiting cell proliferation (i.e., decreasing cell viability) or stimulating cell proliferation. Cree also teaches that "cells can be obtained by dissociating animal or human tissues" (page 171, paragraph 2). Cree discloses that cells can be obtained from blood samples (page 173). On pages 174-175, Cree teaches methods for preparing extracts from cells, for measuring ATP by a luciferase-luciferin assay, and for analysis of the results. In Figure 3 (page 175) Cree demonstrates the use of the methods to determine the effect of chemotherapeutic agents on the proliferation of cancer cells. Cree discloses that cells can be treated with antibiotics (pages 171-172). Cree teaches the necessity of measuring cell proliferation in the absence of the agent so that these data may be compared to data acquired in the presence of the agent (see, for example, Figure 2, page 174). Clearly, Cree's method is intended for use with an ATP-dependent luciferase, such as firefly luciferase, because the assay actually measures ATP rather than luciferase activity, *per se*.

Cree does not expressly disclose a method for determining the effect of an agent on the proliferation of cells containing a *Renilla* luciferase polypeptide or containing a polynucleotide encoding a *Renilla* luciferase, such as a HeLa cell transfected with a transgene encoding *Renilla* luciferase. Also, Cree does not specifically teach that the method can be used to determine proliferation of prokaryotic cells over time.

Virta, et al teach a method that is similar to the method of Cree. However, the method that Virta, et al teach is different because it has been modified to enable determination of the cytotoxicity of an antibiotic agent by directly measuring the proliferation of the cells. In contrast, Cree's method requires that cell proliferation be measured indirectly. Virta, et al teach that prokaryotic cells can be transfected with a transgene that encodes a luciferase for use in the assay (page 2775, column 1 and abstract). Virta, et al teach a method of measuring the luminescence of transfected cells directly (page 2777, column 1) and that there is a correlation between bacterial viability and luciferase activity over time in data collected from cells treated with an antibiotic agent (see for example, Figure 4, page 2777). Virta, et al teach that "expression of the luciferase gene in a target organism makes screening fast and reliable" (page 2778, column 2). Also, in the abstract, Virta, et al teach:

Since luciferase uses intracellular ATP to generate visible light which can be measured from living cells in real time, we wanted to compare the extent to which cell viability parallels light emission. Results from luminescence measurements and CFU counts were in good agreement, and the decrease in light emission was shown to provide a rapid and more sensitive indication of cytotoxicity.

However, Virta, et al do not expressly disclose that the method can be used to determine the proliferation of eukaryotic cells isolated from a tumor in a human subject, such as HeLa cells. Also, Virta, et al does not teach that cells can be transfected with a transgene encoding *Renilla* luciferase, per se.

Edinger, et al teach a method similar to the method of Virta, et al. The method of Edinger, et al differs from the method of Virta, et al because eukaryotic cells are used in the assay. Specifically, Edinger, et al teach that HeLa cells were stably transfected with a vector carrying a transgene encoding firefly luciferase (abstract). It is well known in the art that HeLa cells are human cells that were obtained from a biological sample comprising a tumor and which are grown in a culture. Proliferation of these transfected HeLa cells can be monitored by repeated measurements of luciferase activity over time. Edinger, et al also teach that tumor cells can be labeled by transfection with a transgene encoding luciferase and the proliferation of these labeled tumor cells after the cells were

injected into mice can be monitored *in vivo* (abstract). Edinger, et al disclose that the noninvasive method for assessing tumor cell proliferation in animal models can be used to “accelerate development of novel therapeutic strategies” (abstract), which can include screening candidate chemotherapeutic agents.

However, Edinger, et al do not teach that cells can be transfected with a transgene encoding *Renilla* luciferase, per se. Edinger, et al also do not expressly disclose that the method can be used to screen mammalian cells to determine their susceptibility to an agent, or that the method can be used to determine the effect of an agent on cell proliferation.

Prosser, et al teach that “monitoring of *luc* [luciferase]-marked cells has been achieved by whole-cell luminescence assays, but also through measurement of cell-free luminescence in total protein extracts” and that “the latter approach provides an assay that is independent of cell activity and cellular energy reserves” (page 167, column 1).

US 6,171,809 B1 teaches that “the reaction of renilla luciferase differs from that of firefly luciferase in that the substrate is a different molecule, coelenterazine, rather than the firefly luciferin, and only oxygen is involved” and that “carbon dioxide is produced as with firefly luciferase, but neither ATP nor AMP are required” (column 2, lines 5-14). US 6,171,809 B1 teaches that a method by which an extended glow can be achieved to facilitate measurements of luminescence in samples of cells that express *Renilla* luciferase (column 2, lines 31-36). US 6,171,809 B1 teaches that vectors containing transgenes that encode *Renilla* luciferase can be transfected into mammalian cell lines (column 5, line 66 to column 6, line 2). These transfected cells can be used in the assay to measure luciferase activity (column 6, lines 9-28). In Example 3, US 6,171,809 B1 teaches a method of compound screening using luminometric measurement of *Renilla* luciferase in the transfected cells (column 7).

US 5,292,658 A teaches the cloning of a cDNA molecule encoding *Renilla* luciferase (abstract) and that the cDNA can be cloned into an expression vector (Figure 6). US 5,292,658 A teaches that “the genetic material can be used to produce the enzyme for use as luminescent tags in bioluminescence assays and for other purposes for which such labels are desirable” (column 2, lines 59-62). US 5,292,658 A teaches

that the transgene encoding *Renilla* luciferase can be expressed in prokaryotic cells and the luminescence from samples can be measured (columns 11-12).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Cree in view of the teachings of Virta, et al and Edinger, et al so that measurement of cell proliferation can be made directly by measuring the luminescence of cells or samples derived from cells that express luciferase. It also would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to substitute a transgene encoding *Renilla* luciferase, as taught by US 5,292,658 A, for the transgenes encoding ATP-dependent luciferases used in the method of Virta, et al and Edinger, et al.

One of ordinary skill in the art would have been motivated to modify the method of Cree in view of the teachings of Virta, et al because Virta, et al teach that expression of the luciferase gene in a target organism makes screening fast and reliable and thus provides a rapid and more sensitive indication of cytotoxicity. One of ordinary skill in the art would have been motivated to modify the method of Cree in view of the teachings of Edinger, et al because Edinger, et al teach that a tumor cell transfected with a transgene encoding *Renilla* luciferase can be monitored *in vivo*, thus providing an *in vivo* assesment of the effect of an agent on cell proliferation, which is more reflective of the clinical effect. In order to determine the effect of an agent on cell proliferation by measuring *in vivo* luminescence of intact cells, one would have been motivated to substitute *Renilla* luciferase for an ATP-dependent luciferase in the method of Virta, et al or Edinger, et al, because US 6,171,809 B1 teaches that *Renilla* luciferase does not require ATP as a substrate and this would circumvent the issue that Prosser, et al raise, which suggests that it is desirable to have an assay that is independent of cell activity and cellular energy reserves. In order to determine the effect of an agent on cell proliferation by measuring luminescence of extracts derived from cells, one would have been motivated to substitute *Renilla* luciferase for an ATP-dependent luciferase in the method of Virta, et al or Edinger, et al, because US 6,171,809 B1 teaches that *Renilla* luciferase does not require ATP as a substrate and thus the assay is considerably

Art Unit: 1642

simplified and because US 6,171,809 B1 teaches a method of increasing the duration of the glow to facilitate measurements of *Renilla* luciferase activity.

Conclusions

11. No claims are allowed.

12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephen L. Rawlings, Ph.D. whose telephone number is (703) 305-3008. The examiner can normally be reached on Monday-Thursday, alternate Fridays, 8:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony C. Caputa, Ph.D. can be reached on (703) 308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.


Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Stephen L. Rawlings, Ph.D.

Art Unit 1642

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March 8, 2001


DONNA WOTTLING
PRIMARY EXAMINER